HOST - PARASITOID RELATIONSHIPS BETWEEN THE CARIBBEAN FRUIT FLY, *ANASTREPHA SUSPENSA* (LOEW) AND *BIOSTERES* (=*OPIUS*) *LONGICAUDATUS* ASHMEAD, A BRACONID ENDOPARASITOID

By

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TABLE OF CONTENTS

TABLE OF CONTENTS - continued

LIST OF TABLES

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate Council
of the University of Florida in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy

HOST - PARASITOID RELATIONSHIPS BETWEEN THE CARIBBEAN FRUIT FLY, ANASTREPHA SUSPENSA (LOEW) AND BIOSTERES (=OPIUS) LONGICAUDATUS ASHMEAD, A BRACONID ENDOPARASITOID

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Biosteres (=Opius) longicaudatus Ashmead, a solitary endoparasitoid was introduced into Florida to suppress populations of the Caribbean fruit fly, Anastrepha suspensa (Loew). B. longicaudatus is being mass-reared on A. suspensa in anticipation of inundative field releases. Aspects of the reproductive biology and host discrimination ability of female parasitoids, as well as the optimum host age for parasitoid development were investigated.

Five-day-old female parasitoids distinguished parasitized larvae from healthy ones within 24 hr of initial parasitization. At the lowest host : parasitoid ratio (6.3:1), the discriminating ability of females was masked but became evident at higher ratios of 14.6:1 and 29.2:1. At the lowest ratio, a mean of 13.1 eggs were deposited per female per day, compared with 13.7 and 23.3 eggs at 14.6:1 and 29.2:1 host:parasitoid ratios, respectively. Although individual females laid fewest eggs at the lowest host : parasitoid ratio, a

mean of 2.10 eggs were deposited per host. This superparasitized condition resulted in high host and parasitoid mortality which was evidenced by a low parasitoid emergence of 28.5% per female per day, compared with 71.5 and 73.0% emergence at 14.6:1 and 29.2:1 ratios, respectively.

Third instars of A. suspensa (5-days-old) were the optimum hosts for parasitoid development at 26°C and 60% RH. Seven-day-old larvae (late 3rd instar) were less suitable. Mean duration of parasitoid egg stage was 2.0 and 4.6 days in 5 and 7-day-old hosts, respectively ($p < 0.05$). The pupal stage was significantly prolonged from 6.4 days in 5-day-old hosts to 7.5 days in 7-day-old hosts. Consequently, the life cycle of the parasitoid was longer (22.6) for those which developed in 7-day-old hosts than for those in 5-day-old hosts (18.5 days). High parasitoid mortality which occurred at the egg and pupal stages in 7-day-old larvae resulted in a lower percent emergence (33.9%) compared with 65.1% from 5-day-old hosts. Although 7-day-old larvae proved relatively unsuitable for parasitoid development, male and female progeny were larger (2.80 and 3.85 mm, respectively) than those from 5-day-old hosts (2.50 and 3.65 mm, respectively). The female:male ratio of progeny from 7-day-old hosts was 2.37:1 and 1.0:1.87 for 5-day-old larvae.

Ligation studies showed that 5-day-old A. suspensa larvae had not yet reached the critical period. Seven-day-old larvae (late 3rd instar) had passed the critical period. Five-day-old larvae were injected or topically treated with Altozar$^{(R)}$ 50%, a juvenile hormone analogue(JHA), at a dosage of 2.77 µg JHA per gram of larva. Fifty-eight percent of topically treated and 47% of injected larvae

took 8 and 9 days, respectively to pupate. Seventy-nine percent of untreated larvae pupated in 7 days. Durations of the parasitoid egg stage in injected, topically treated and untreated 5-day-old hosts, were not significantly different. Percent parasitoid emergence from topically treated hosts was higher ($p < 0.05$) than that from injected or untreated 7-day-old larvae. The trauma of injection caused high mortality. Of all larvae tested 5-day-olds were the most suitable.

Five-day-old B. longicaudatus females laid 20-30 eggs per day, given an adequate supply of hosts. Females are 'synovigenic' and maximum egg laying potential of mated females is achieved within 5 days of emergence. Mated females with 72 hr oviposition experience matured eggs faster than those with 24 hr oviposition experience.

CHAPTER I

GENERAL INTRODUCTION

The Caribbean fruit fly, Anastrepha suspensa (Loew)[1] (Fig. 1) is a pest of numerous tropical and subtropical fruits in Cuba, Jamaica, Hispaniola, Puerto Rico, and Southern Florida (Weems 1965). Swanson and Baranowski (1972) reported that the first occurrence of the fruit fly in Florida was in 1931. Two flies were caught in Key West, Florida in 1959 and larvae were discovered in fruit in Miami Springs, Florida in 1965 (Weems 1965). Swanson and Baranowski (1972) indicated that this tephritid attacked 84 host fruits in 23 families in Florida. Among these 84 hosts were 11 species or cultivars of Citrus. Most of the Citrus attacked were overripe fruit in backyard gardens and infestation was low (Swanson and Baranowski 1972). Nevertheless, the fact that A. suspensa attacked species of Citrus made it a potential pest to commercial citrus in Florida. This prompted an investigation of possible factors that could lend themselves to successful control programs against the caribfly.

The use of insecticides other than bait sprays for control of the fruit fly has been contemplated but due to the fact that larvae burrow into the fruit, effective control would be difficult if treated fruit are to be edible. The use of beneficial insects is a promising

[1] Diptera : Tephritidae.

Figure 1. Female of Anastrepha suspensa ovipositing in guava fruit.

alternative method of control. It was to this end that the importation of parasitoids was initiated in 1967 (Baranowski and Swanson 1971). Of the 10 species of parasitoids introduced, Parachasma cereum Gahan[2] and Biosteres (=Opius) longicaudatus Ashmead[2] were the most promising (Baranowski, personal comm.).

Before parasitoids can be used effectively in inundative release programs for pest control, efficient mass-rearing procedures must be developed. To achieve this, basic biological information should first be obtained on the target pest and the parasitoid, and their interrelationships should be understood. B. longicaudatus was selected for this study. This parasitoid is free living as an adult but passes its immature life within larvae and puparia of its tephritid hosts (Clausen, Clancy, and Chock 1965), which it eventually destroys. Thus it is a protelean parasitoid in that only the immature stages are parasitic (Askew 1971).

The Principle of Host Selection

The relationship between a host and its potential parasitoid begins with a series of behavioral processes initiated by the parasitoid. These host selection processes were first classified by Salt (1935, 1938) as (1) ecological selection (2) psychological selection and (3) physiological selection. Doutt (1964) divided Salt's first category into (1) host habitat finding and (2) host finding. Doutt (1964) also converted Salt's remaining steps to (3) host acceptance and (4) host suitability. To these 4 steps, Vinson (1975) has added a fifth, called host regulation. To Vinson (1975), the first 3 steps comprise host selection and the

[2]Hymenoptera : Braconidae

remaining 2 describe factors which result in successful parasitization. The first 3 sequential processes are achieved through the sensory mechanisms of the parasitoid in which chemical and physical cues from the host and/or the habitat help to direct the orientation of the female parasitoid, thus restricting the habitats searched. As each host selection process is completed, the list of potential hosts narrows. The final process results in the encounter of hosts within which the parasitoid may be able to successfully develop. The above mentioned pathways of host location culminate in a "find and attack cycle" as summarized by Lewis, Jones, Norlund, and Gross (1975).

Host Habitat Finding

This aspect of the host selection process is related to the preference of the parasitoid for a particular type of environment. Habitat preference may be the selection of suitable temperature, humidity, light intensity or other physical factor (Doutt 1964). For example, Vinson (1975) showed that Cardiochiles nigriceps Viereck, a parasitoid of Heliothis virescens (F.), occurs in open fields and pastures but not in forested or heavily shaded areas (Vinson 1975). Similarly, high light intensity is required for successful laboratory rearing of this species (Vinson, Guillot, and Hays 1973).

Before a relationship can be initiated, the potential host and parasitoid must be ecologically coincident. Hence, potential hosts which might be suitable but occur outside the parasitoid's preferred habitat are ignored. In an extensive review of host selection, Vinson (1976) stated that the parasitoid is directed by

a hierarchy of chemical and physical cues associated with the host. The parasitoid may be attracted by the sight of the food plant of its potential host. For example, the ichneumonid, Apechthis rufata Gmel. normally attacks pupae of the oak tortricids, Tortrix viridana (L.) and Archips xylosteana L. (Zwolfer and Kraus 1957). However, when pupae of the fir budworm, Choristoneura muriana Hubner were artificially placed in leaf rolls of oak, A. rufata females parasitized them but ignored the same fir budworm pupae naturally occurring on fir trees close by (Zwolfer and Kraus 1957).

Chemicals emanating from the food source of the host may also attract the parasitoid; e.g., Horogenes chrysotictos Gmelin, a parasitoid of certain flour moths, was attracted to the odor of oatmeal or flour regardless of the presence of hosts (Fisher 1959). Mature females of the ichneumonid, Pimpla ruficollis Grav., a parasitoid of the pine shoot moth, Rhyacionia buoliana Schiff, were strongly attracted to the odor of oil from the host plant, Pinus sylvestris (Thorpe and Caudle 1938). Color may be among the factors that attract a parasitoid to its hosts. Arthur (1966) showed that Ictoplectis conquisitor (Say) females can be conditioned to associate color with the presence of hosts. Vinson (1976) reviewed the role of physical and chemical cues in orienting the parasitoid toward its host. He concluded that the responsiveness of many hymenopterous parasitoids to plant odors may stem from an originally plant-parasitic habit.

Host Finding

Parasitoids can distinguish a series or hierarchy of cues which

lead to the host. For example, having located the host plant, the parasitoid may detect odors such as those resulting from plant tissue damaged by the host. Hassell (1968) found that the tachinid, Cyzenis albicans (Fall.), located the winter moth, Operophtera brumata (L.) by detecting sugars released from the damaged plant leaves.

Short-range host cues are usually associated with the presence of the host and are host-indicator substances or kairomones. Kairomones by definition, are "transpecific-chemical messengers, the adaptive value of which falls on the recipient rather than the emitter" (Brown, Eisner, and Whittaker 1970). These short-range messengers elicit extensive searching behavior by the parasitoid. The chemical identity of only a few kairomones is known (Vinson 1975). Pheromones of the host species may also be used as kairomones by parasitoids; e.g., species of Aphytis are attracted by the pheromone of their host, the California red scale, Aonidiella auranti (Maskell) (Sternlicht 1973). The many factors which may contribute to host finding are reviewed by Vinson (1976).

Host Acceptance

Having located the host, the parasitoid must first receive certain stimuli before the potential host is recognized as being suitable, and oviposition occurs. Factors which may be important in host identification or recognition include odor, movement, sound, and electromagnetic radiation (Vinson 1975). The presence of host-seeking stimulants may work in conjunction with some of the aforementioned physical cues. Substances such as amino acids and sugars in the hemolymph of the lepidopterous host of Ictoplectis

conquisitor stimulated the parasitoid to oviposit (Arthur, Hegdekar, and Rollins 1969).

A second aspect of host acceptance, host discrimination, may occur when the parasitoid distinguishes between already parasitized and healthy hosts. In the case of parasitoids which can discriminate, deterring substances are detected by the female parasitoid on or within already parasitized hosts. Salt (1934) was the first to indicate that host eggs contaminated by a Trichogramma evanescens Westwood female were no longer acceptable to other conspecific females. Salt also found that these deterring substances could be removed by washing, thus reinstating the superficial acceptability of the host. These "spoor factors" or marking pheromones, when perceived by other parasitoids of the same species help prevent superparasitization, which is the occurrence of more larvae of a single parasite species than can mature in that host (Doutt 1964).

Some parasitoids are so responsive to kairomones normally associated with their usual hosts, that hosts which would be otherwise unacceptable may be accepted after treatment with kairomones from the normally attractive hosts; e.g., Greany (1971) induced oviposition into physiologically inappropriate hosts by Orgilus lepidus Musebeck with the use of frass of the potato tuberworm, Phthorimoea operculella (Zeller). This material contained host-seeking stimulants naturally associated with P. operculella, the normal host of the parasitoid.

Host Suitability

According to Vinson (1975) this process is not akin to the behavioral steps of the host selection processes, but like host

regulation, refers to the physiological interactions of the host and parasitoid. Once parasitized, the host must be suitable for parasitoid development (Vinson 1975). Suitability depends on the nutritional and physiological state of the host. For example, Smilowitz (1974) showed that successful development of Hyposoter exiguae (Viereck) occurred in larvae of Trichoplusia ni Hubner which had not yet reached the critical period during which molting hormone is secreted in preparation for pupation.

Host suitability is related to the size of the parasitized host; e.g., Salt (1941) showed that progeny of T. evanescens became runts or individuals with improportionate appendages when reared on very small lepidopterous host eggs. Host suitability is also dependent on the ability of the parasitoid to overcome the defense mechanisms of the host as exemplified by Pseudeucoila bochei Weld which is thought to inject a chemical into its host, Drosophila melanogaster, preventing encapsulation of the simultaneous parasitoid Pseudeucoila millipes (Streams and Greenburg 1969). In cases of multiple or superparasitism, the parasitoid must also be able to successfully compete with other parasitoids. Multiple parasitism is defined by Doutt (1964) as the "simultaneous parasitization of a single individual host by 2 or more different species of primary parasites." Pemberton and Willard (1918) showed that superparasitization by Diachasma tryoni resulted in first instars destroying each other with their mandibles. The weaker, damaged larvae become encapsulated by the host. Other parasitoids physiologically suppress their competitors by secretion of toxic substances, or depletion of the oxygen supply (Fisher 1971).

Host Regulation

This fifth stage recognized by Vinson (1975) emphasizes the role of the parasitoid in changing the host's development to facilitate its own development. Parasitized larvae of H. virescens grew slower than unparasitized larvae when attacked by C. nigriceps (Vinson and Barras 1970). Similarly, Johnson (1959) showed that Aphidius platensis Brethes delayed the maturation of its host, Aphis craccivara Koch.

Host regulation may also result from the ability of the parasitoid to regulate the internal environment of its host. Dahlman and Vinson (1976) found that the trehalose level in the blood of hosts parasitized by C. nigriceps was decreased while that of hosts parasitized by Microplitis crociepes Cresson was elevated. This increase in carbohydrate reserve was thought to have been more important to M. crociepes which is a hemolymph feeder (Vinson 1975).

Objectives of This Dissertation

One of the main purposes of the investigations outlined herein was to become familiar with basic interrelationships of B. longicaudatus and A. suspensa to allow refinement of mass rearing of this parasitoid. The parasitoids so produced would be intended for use in field releases against the fruit fly.

The ability of the parasitoids to discriminate previously-parasitized hosts from non-parasitized hosts was determined, as well as the effect of host availability upon superparasitization. This effort was made to optimize the rearing success of parasitoids.

Additional studies were performed to establish the optimum host stage for parasitoid attack and development so as to further refine the mass rearing program.

An effort was made to prolong the optimum stage by injecting host larvae with a juvenile hormone analogue. With the optimum larval stage prolonged, the suitability of the host could also be prolonged and the developmental success of the parasitoid possibly increased. Aspects of the reproductive biology of female parasitoids were investigated to determine whether females emerged with their full complement of eggs ('proovigenic') or whether eggs were matured at intervals in the life of the female ('synovigenic'). In the latter case, egg production could be related to the female parasitoid's diet (Doutt 1964). Hence, information on the reproductive biology could be valuable in defining a diet that would facilitate high rates of egg production by female parasitoids.

CHAPTER II

GENERAL REARING METHODS AND DEVELOPMENTAL BIOLOGIES OF A. SUSPENSA AND B. LONGICAUDATUS

Introduction

Members of the genus Opius are known to parasitize tephritid fruit flies, including Anastrepha species, in nature (Clausen et al. 1965). A. suspensa, though not the original host of Biosteres (=Opius) longicaudatus, has served as an adequate laboratory host for this parasitoid (Greany, Ashley, Baranowski, and Chambers 1976). The colonies of A. suspensa and B. longicaudatus used in these studies were initially obtained from colonies started in Homestead, Florida by Dr. R. M. Baranowski.

Materials and Methods

Rearing of A. suspensa

Fruit fly adults were fed yeast hydrolysate and sucrose crystals. Water was also provided via a dental wick from a small reservoir. Females oviposited through a wax covered gauze panel to the outside where the eggs were collected.

Eggs were transferred to a sugar cane bagasse diet developed by R. M. Baranowski (Greany et al. 1976), which served as larval food. Details of rearing methods of A. suspensa are given by Burditt, Lopez-D, Steiner, Von Windeguth, Baranowski, and Anwar (1975). Mature larvae were allowed to crawl from the expended diet, through 0.3cm screen mesh into moist vermiculite (50% water content, W : W).

Rearing of B. longicaudatus

This parasitoid attacks the larval stages of the fruit fly. Young 3rd instars (4-5 days old) reared as described above, were utilized as hosts. Larvae confined within 13.5cm diameter oviposition units described by Greany et al., (1976), were exposed to B. longicaudatus females in a $25cm^3$ plexiglas cage for 24 hr. Adult parasitoids were fed honey and water.

After the oviposition period, host larvae were removed from oviposition units and put into moist vermiculite (50% water content, W:W) to pupate. Adult parasitoids which emerged were used as test animals in subsequent experiments.

Developmental Biology of A. suspensa

Initial observations indicated that the developmental stage reached by A. suspensa larvae at the time of parasitization had some effect on the success of parasitoid development. Since there was no information in the literature on the number of A. suspensa larval stages and their duration, it became necessary to determine the number of larval stages, their duration and characters which could be used to distinguish them. Specimens used were reared as described in "Rearing of A. suspensa," the only exception being a more constant temperature of 26°C and a relative humidity of 60%. No later than 12 hr. after eclosion, 20 larvae were removed from the medium and preserved in 70% EtOH for further observation. Subsequent samples of 20 larvae each were preserved every 24 hr. until pupation occurred. Each age group was replicated 3 times.

Before mouth hooks were measured, larvae 3 days and older were slit longitudinally and put in a 10% KOH solution to clear overnight.

The mouth hooks and cephalopharyngeal skeleton were removed and mounted in Hoyer's mounting medium. The last abdominal segment bearing the posterior spiracles was severed and mounted in the manner described by Phillips (1946). Younger larvae were mounted directly in Hoyer's, intact.

The mouth hooks of all larvae were measured using an eye-piece micrometer. The last abdominal segment and the rest of the integument were observed for characters that could be used to separate each instar.

Developmental Biology of B. longicaudatus

Since B. longicaudatus is an endoparasitoid, a determination of its larval stages and their duration had to be done by dissection of the parasitized hosts. Host larvae were exposed for 24 hr to parasitization by B. longicaudatus females, and were then put in moist vermiculite to pupate. Samples of parasitized hosts were put in 70% EtOH at the end of the 24 hr oviposition period and on each succeeding day until parasitoids began to emerge.

Results and Discussion

Developmental Biology of A. suspensa

Duration of the immature stages varies with temperature. Prescott and Baranowski (1971) found that the mean incubation periods of A. suspensa eggs at 15, 20, 25 and 30°C were 243.4, 106.5, 73.1 and 57.0 hr, respectively. They also found that the mean pupation times at 15, 20, 25 and 30°C were 29.1, 15.7, 8.9 and 7.8 days, respectively. However, they did not present the duration of the larval stages. The duration of the egg stage at 26°C was found to be 48-72 hr in the

present study. The 1st and 2nd stadia each lasted from 1-2 days, while the third and final instar pupated in 3-4 days. The duration of the pupal stage was 14-15 days. I observed that the duration of the last larval stage was prolonged if the bagasse diet was too moist and larvae were confined with no option of crawling to a drier environment.

The morphology of the mouth hooks provided a reliable means of distinguishing A. suspensa instars. Measurements of the mouth hooks (Table 1) also confirmed that there are 3 instars. Statistical analysis by Duncan's new multiple range test showed that the mean lengths of the 3 types of mouth hooks were significantly different ($p < 0.01$). The morphology of the mouth hooks is shown in Fig. 2. The mouth hooks of the 1st instar (Fig. 2A) were lightly sclerotized and those of newly eclosed larvae were pink while older 1st instars (1-2 days) were yellowish. Larvae in the late 1st instar (2 days old) had both type A and B mouth hooks. Functional 1st instar mouth hooks (Fig. 2A) protruded from the anterior end of the larvae, while the 2nd set of faintly sclerotized mouth hooks (Fig. 2B) was located within the head just behind and dorsal to the functional 1st instar mouth hooks. Early 2nd instars had type B mouth hooks only (Fig. 2), while late 2nd instars had both type B and C (Fig. 2). The latter was posterior and dorsal to the functional 2nd instar mouth hooks (Fig. 2B). Only type C (Fig. 2) was observed in larvae 5 days of age and older. These were the most heavily sclerotized mouth hooks and there was no median tooth as in the 2 earlier instars. Type C was also dissected from puparia of A. suspensa, indicating that this was the final type exhibited.

Table 1.--Determination of number of instars of Anastrepha suspensa on the basis of mouth hook measurements

Age of Larvae (days)	Length (u) of larval mouth hook			
	n	Range	Mean*	SE
1-2	60	37 - 40	39.20[a]	0.257
3-4	60	140 - 145	141.75[b]	0.547
5-6	60	210 - 225	217.75[c]	1.147
7	60	220 - 230	225.00[c]	1.147

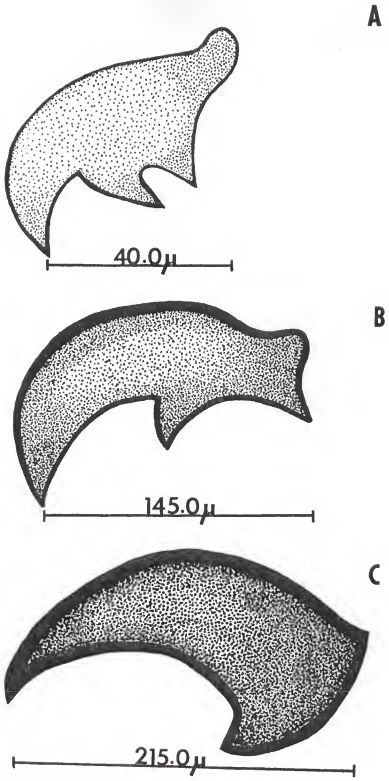
*Numbers in the same column followed by the same letter are not significantly different at the 0.01 level by Duncan's new multiple range test.

Figure 2. Morphology of the mouth hooks of the instars of Anastrepha suspensa.

A. Mouth hook of 1st instar

B. Mouth hook of 2nd instar

C. Mouth hook of 3rd instar


A
40.0μ
B
145.0μ
C
215.0μ

Mouth hooks were the most reliable indicators of larval stages of the caribfly. Other workers have used mouth hooks and the cephalopharyngeal apparatus for identification of some tephritid species (Phillips 1946), for identification of tephritid larval stages (Kamali and Schelz 1973), and for separation of instars of Drosophila (Bodenstein 1965). Examination of the larval integument and last abdominal segment did not reveal any characters that readily distinguished instars.

Developmental Biology of B. longicaudatus

Eggs of B. longicaudatus were deposited singly, between the integument and intrasegmental muscles of the host. Newly laid eggs were transparent and ellipsoidal. The short pedicels were cemented to the inner wall of the integument. The site of oviposition eventually darkened, leaving a readily discernible oviposition scar.

Eggs laid in early 3rd instar hosts usually hatch in 2 days but the time of eclosion was found to be prolonged when mature (6 and 7-day-old) host larvae were exposed.

The 1st stadium lasted 2 days, and the newly eclosed larva had a mass of serosal cells attached to the ventral surface similar in appearance to that described for Diachasma tryoni Cameron by Pemberton and Willard (1918), Opius humilis Silvestri, and O. fullaway Silvestri (Clausen 1940).

Supernumary 1st instars attack each other with their powerful mandibles and only one survives to the second instar. Dead larvae are encapsulated by the host. The 2nd and 3rd stadia each lasted 2 days while the 4th (final) instar had a duration of 4 days. The

pupal stage lasted 6 days but may be prolonged if the host is parasitized at 6-7 days of age.

Total developmental time for females was 19-23 days and 18-22 days for males. This period is dependent on the age of the host at the time of parasitization.

CHAPTER III

HOST DISCRIMINATION IN INSECT PARASITOIDS

Introduction

The term host discrimination has been used by some workers (Weseloh 1970, Calvert 1973) to refer to the ability of a parasitoid to differentiate hosts of different species, size, shape, odor or state of health. Other workers (Fisher 1960, 1961 and 1971; Greany and Oatman 1972; Vinson 1975) refer to host discrimination as the ability of a parasitoid to distinguish between parasitized and non-parasitized hosts. This latter definition will be used in this dissertation also. Salt (1934) first demonstrated host discrimination when he noticed that Trichogramma evanescens eggs were not laid entirely at random; i.e., more hosts than expected possessed only 1 parasitoid egg, and fewer than expected possessed >1. Many examples of the host discriminating ability of parasitoids have been demonstrated (Vinson 1975). Some parasitoids have been shown to be capable of host discrimination, but when provided with too few hosts, may superparasitize them but lay fewer eggs than normal; e.g., Salt (1936) found that females of Trichogramma evanescens restrained themselves when too few hosts were provided, and oviposited only 5% of their available eggs.

Vinson (1975) stated that host selection by parasitoids is mediated by a series of external and internal chemical cues detected

by the antennae and ovipositor of parasitoids. Salt (1934) first demonstrated that washing parasitized eggs of Sitotroga removed the external marking chemical left by the parasitoid which first oviposited therein. However, when the parasitized egg was penetrated by the ovipositor of a second parasitoid, the host egg was rejected. Fisher and Ganesalingam (1970) have shown that changes in the free amino acid composition of the hemolymph of Ephestia kuehniella Hubner larvae occurred after parasitization by Nemeritis canescens Grav. These workers suggested that it may have been these changes in hemolymph composition that were detected by the ovipositor of the parasitoid, resulting in avoidance. Electrophoretic studies by King and Rafai (1970) showed that there was a protein in the hemolymph of parasitized Nasonia vitripennis (Walk.) pupae which was absent in healthy hosts.

Some workers attribute the source of oviposition deterrents to substances injected with the parasitoid's egg into the host's hemolymph; e.g., Guillot and Vinson (1972a) showed that secretions from Dufour's gland mediated host discrimination by Campoletis perdistinctus. Others (Fisher 1971) have shown that discrimination was more evident in hosts that contained advanced stages of the parasitoid than in hosts with only parasitoid eggs present. Vinson (1975) reviewed the possible means by which parasitoids may detect external and internal marking substances. It is possible that there may be a variety of secretions from different sources mediating discrimination, depending upon the species concerned. The mechanisms which mediate discrimination were not investigated for B. longicaudatus but preliminary observations suggest that they might be similar to

those of Cardiochiles nigriceps reported by Guillot and Vinson (1972b).

Demonstration of Host Discrimination in B. longicaudatus

Biosteres longicaudatus is a solitary endoparasitoid (Clausen et al. 1965). There is no reference in the literature concerning the ability of the females to discriminate.

The fact that availability of hosts can affect the number of eggs laid by female parasitoids (Doutt 1964, Fusco and Hower 1974) and the possible significance of discrimination towards refinement of a mass rearing program, led to further investigation of the oviposition behavior of B. longicaudatus.

Materials and Methods

Five-day-old (early 3rd instar) A. suspensa larvae were used in studies on host discrimination. Three host population sizes (150, 350 and 700 larvae) were exposed to 24 pr of 5-day-old B. longicaudatus (host : female parasitoid ratios of 6.3:1, 14.6:1 and 29.2:1 respectively).

The parasitoids were put in 25cm^3 plexiglas cages and were fed honey and water. Larvae were exposed for 24 hr to the parasitoids in oviposition units similar to those described by Greany et al., (1976), except that they were 4cm in diameter. Each host : parasitoid ratio was replicated 3x. All parasitization occurred at 26°C and 60% RH.

After the 24 hr exposure period, parasitized larvae were held for an additional day to allow for parasitoid egg enlargement to increase visibility. At the end of this period 50 larvae from each

group were preserved in 70% EtOH for later dissection. The remaining larvae were allowed to continue development until parasitoid emergence. This facilitated a determination of the effect of host:parasitoid ratios on percent parasitization (=the ratio of the number of emerged parasitoids to the number of puparia x 100). Occurrence of female progeny confirmed mating by test females. Upon dissection, note was made of the number of eggs found per host and this egg distribution was compared with a theoretical random (Poisson) distribution (Wadley 1967), using a chi-square analysis. Significant differences between the actual and calculated random distributions were considered to be an indication of discrimination by B. longicaudatus females.

Results and Discussion

The highly significant ϕ^2 values in Table 2 indicate that B. longicaudatus females oviposited in a discriminatory, non-random manner. The non-significant ϕ^2 values obtained for the lowest host:parasitoid ratio (6.3:1) indicate that females were deprived of sufficient hosts and this stressful condition masked any manifestation of the parasitoids' discriminatory ability. The unavailability of sufficient acceptable hosts could give results which might cause one to arrive at the erroneous conclusion that the parasitoid was unable to discriminate. The females presumably detected the parasitized condition of the hosts but oviposited nevertheless since the availability of acceptable hosts was restricted. For example, Hays and Vinson (1971) concluded that Cardiochiles nigriceps could not distinguish between parasitized and non-parasitized Heliothis virescens larvae because they offered

Table 2.--Egg Distribution by 24 mated, 5-6-day-old Biosteres longicaudatus females at different host:parasitoid ratios

Replicate	No. host larvae with n eggs					No. parasitoid eggs dissected		
	0	1	2	>3	Total	$\bar{x}$/larva	Total	ϕ^2value
				150 larvae exposed (6.3:1)+				
A	4	15	16	15	50	2.06	103	1.82^{ns}
B	6	7	19	18	50	2.10	105	4.93^{ns}
C	6	8	20	15	50	2.16	108	4.98^{ns}
				350 larvae exposed (14.6:1)+				
A	12	29	8	1	50	0.96	48	10.17**
B	15	33	2	0	50	0.74	37	22.16**
C	8	28	14	0	50	1.12	56	10.93**
				700 larvae exposed (29.2:1)+				
A	17	27	6	0	50	0.78	39	8.21*
B	13	32	5	0	50	0.84	42	35.62**
C	14	32	4	0	50	0.80	40	17.45**

ns, *, ** Distribution of eggs not significantly different from, and significantly different (at the 5 and 1% levels respectively) from a Poisson distribution using x^2 analysis.

+Numbers in parentheses represent host:parasitoid ratios.

only one host at a time to the parasitoids. However, Guillot and Vinson (1972b) later showed in choice tests that this species can discriminate. Earlier work by Chamberlain and Tenet (1926) had also indicated that Cardiochiles nigriceps could discriminate.

There was evidence of restraint in the number of eggs laid per female at the lower (6.3:1 and 14.6:1) host:parasitoid ratios (Table 3). More eggs were laid at the highest (29.2:1) ratio (Table 3). This ability of female parasitoids to exercise restraint in the number of eggs laid, reduces egg wastage since only one parasitoid can develop per host. This also decreases the degree of host mortality likely to occur from stress through superparasitization (Fusco and Hower 1974). Restraint has been observed by Fisher (1961) in females of Horogenes chrysostictos and Nemeritis canescens, which are parasitoids of mature larvae of Ephestia sericarium. Kusano and Kitano (1974) also observed that Apanteles glomeratus L. laid significantly fewer eggs in already parasitized larvae of Pieris rapae crucivora than in healthy hosts.

When hosts were exposed to parasitoids in the ratios of 14.6:1 and 29.2:1, 83% and 90% respectively, received either 0 or 1 egg per larva. This resulted in an increase in the number of progeny (9.8 to 16.8, respectively) per female per day (Table 3). The percent survival of progeny was also increased (Table 3, Fig. 3), concomitant with the low rate of superparasitization. However, at the highest host:parasitoid ratio (29.2:1), a mean of 68 hosts received no parasitoid eggs, resulting in emergence of flies, thus affecting

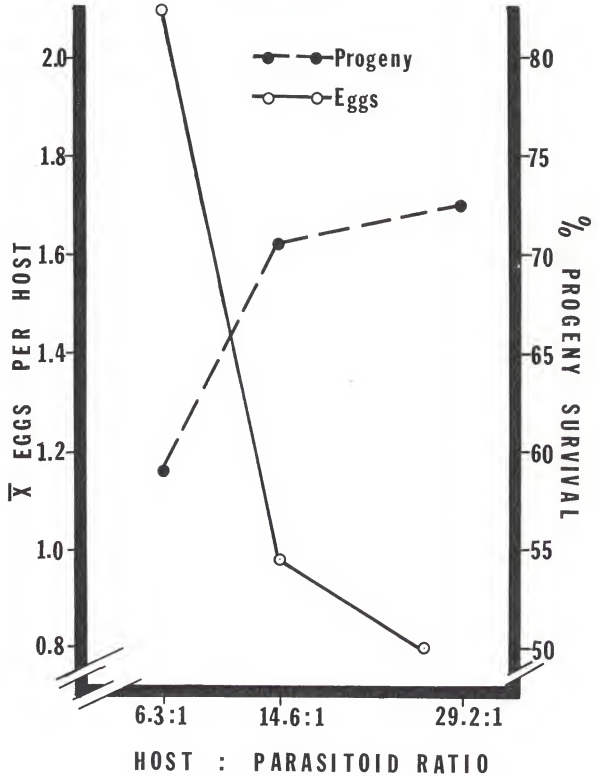
Table 3.--Effect of host : parasitoid ratio on mean oviposition rate and successful development of progeny of Biosteres longicaudatus

No. hosts exposed*	$\bar{x}$ eggs deposited / ♀ / day	$\bar{x}$ progeny produced/♀/day	$\bar{x}$ % progeny/♀/day reaching adulthood
150 (6.3:1)+	13.1	3.7	28.5
350 (14.6:1)+	13.7	9.8	71.5
700 (29.2:1)+	23.3	16.8	73.0

*Mean of 3 replicates each exposed to 24 5-6-day-old B. longicaudatus females and their male partners.

+Numbers in parentheses represent host : parasitoid ratio.

Figure 3. Effect of host:parasitoid ratio on mean egg distribution per host and percent emergence of Biosteres longicaudatus progeny.


Progeny
Eggs
2.0
1.8
1.6
1.4
1.2
1.0
0.8
$\bar{X}$ EGGS PER HOST
80
75
70
65
60
55
50
% PROGENY SURVIVAL
6.3:1
14.6:1
29.2:1
HOST : PARASITOID RATIO

percent survival calculations. Since a mean of only 5 flies emerged at the lower ratio (14.6:1), the percent survival at these 2 host: parasitoid ratios (29.2:1 and 14.6:1) did not differ proportionately (Table 3, Fig. 3). Thus based on percent survival (Table 3, Fig. 3), it may be more economical to use the host:parasitoid ratio of 14.6:1 since half as many hosts gave progeny survival rates similar to those of the highest host:parasitoid ratio.

The inverse relationship between mean number of parasitoid eggs per larva and the percent survival observed for B. longicaudatus at different host:parasitoid ratios was also observed by Fusco and Hower (1974) in Microtonus aethiops (Nees), a parasitoid of Hypera postica (Gyllenhal), the alfalfa weevil. The high mortality associated with limited host availability was attributed to stress from super-parasitization (Fusco and Hower 1974), and this presumably was the case with B. longicaudatus also.

B. longicaudatus females were able to discriminate among already parasitized and non-parasitized hosts within 24 hr of initial oviposition; i.e., during the egg stage of the host. By capitalizing on the ability to differentiate newly parasitized hosts from healthy ones, we now have an additional tool which could be used for further refining the mass rearing program of B. longicaudatus. Detection of young parasitoids (eggs) within a host decreases the problem of superparasitization and high mortality due to competition among supernumaries. The determination of an optimum host:parasitoid ratio will also help to reduce the cost of mass rearing since initially there was a tendency to provide too few or too many hosts per parasitoid female.

CHAPTER IV

HOST SUITABILITY IN INSECT PARASITOIDS

Introduction

Salt (1938) describes a suitable host as one on or in which a parasitoid can produce fertile offspring. Thus the two prerequisites for host suitability are as follows: (a) having accepted a host, the parasitoid must be able to oviposit successfully on or in it, and (b) after parasitization, the progeny must be able to develop successfully in the host (Salt 1938). In this dissertation discussion of host suitability will deal only with the ability of parasitoids to successfully develop within their hosts. In order to be suitable, a host must meet all physical and chemical requirements of each stage of the parasitoid (Salt 1938). Once these requirements are met, a unique relationship develops, resulting in a high degree of specificity and the list of potential hosts is thus reduced. Physical unsuitability may be related to the size of the host or the consistency of its body tissues. The size of a host can affect the size of its parasitoids; e.g., Salt (1941) demonstrated that the offspring of Trichogramma evanescens increased in size with the size of the lepidopterous egg on which they were reared. The effect of a host on the morphology of its parasitoid was further demonstrated when Salt (1937) reared apterous males of Trichogramma semblidis (Aurivillius) from eggs of the alder fly, Sialis lutaria L. while

those from lepidopterous hosts had wings. Arthur and Wylie (1959) found that the weight of Pimpla turionellae (L.) increased with the weight of its hosts. Askew (1971) pointed out that dimorphism of parasitoids can also result from differences in the consistency of the hosts' tissues. He reviewed the work of Schmieder in which offspring of Melittobia chalybii Ashm., a chalcid parasitoid of honeybee larvae had well developed wings and eyes when they fed on solid tissues of the host, but those which fed mainly on body fluids, had reduced wings.

The host serves as both food and physical environment for the parasitoid. Consequently, chemical unsuitability of the host may affect the parasitoid physiologically (Salt 1941). Bradley and Arbuthnot (1938) found that development of Chelonus annulipes Wesmael ceased if its host entered diapause, and the parasitoid would not develop beyond the 1st instar until its host was about to pupate. Smilowitz and Iwantsch (1973) determined that the rate of development of Hyposoter exiguae, a parasitoid of Trichoplusia ni, was faster in older hosts. Increased host age had the opposite effect on Campoletis sonorensis which developed in larvae of Heliothis virescens (Vinson 1972). Arthur and Wylie (1959), like Vinson, found that Pimpla turionellae developed faster in small and medium-size lepidopterous pupae than in larger ones.

The sex ratio of many hymenopterous parasitoids has been observed to vary with the size of the host. The male:female ratio of Pimpla turionellae offspring was high when hosts weighed less than 0.113 gm. (Arthur and Wylie 1959). Taylor and Stern (1971) observed that parasitoid oviposition in small, old or otherwise

less suitable host eggs, resulted in a higher female:male ratio of Trichogramma semifumatum. Brunson (1937, 1938) determined that more male progeny of Tiphia popilliavora Roh. emerged from 2nd instar Popillia japonica Newm., than females. The 3rd (final) instars which were 2x as large as 2nd instars, gave rise to more female than male parasitoids (Brunson 1937, 1938). The sex of parasitoid progeny is determined by the female at the time of oviposition and may depend on the kind of host ('psychological selection'). Sex ratio may also depend on differential survival of the sexes within the host ('physiological selection').

The developmental and physiological states of the host and their effect on the parasitoid have been investigated more closely by a few workers. Lewis and Redlinger (1969) found that oviposition and development of Trichogramma evanescens in eggs of Cadra cautella (Walker) were less successful during the last 12-24 hr of incubation, when the features of the heads of host embryos were evident. Smilowitz (1973) observed that prior to the 5th instar, Trichoplusia ni larvae were most suitable for development of Hyposoter exiguae, early 5th instars were less suitable and late 5th instars were completely unsuitable. Ligation studies (Smilowitz 1974) showed that production of molting hormone in the late 5th instar coincided with larval unsuitability for parasitoid development. Cals-Usciati (1969) also showed that there was a relationship between the production of molting hormone in larvae of Ceratitis capitata Weid. and the development of Opius concolor Szepl. beyond the 1st instar.

In the following investigation, an effort was made to determine the most suitable stage of A. suspensa larvae in terms of its effect

on the size, sex ratio, time of development and number of progeny of B. longicaudatus produced. These studies were followed by an analysis of the relationship of the endocrinological status of the host and its suitability for development of B. longicaudatus.

Parasitoid Emergence as an Indicator of Host Suitability

Materials and Methods

A choice experiment to determine host age preference was conducted. Five cages were prepared and each contained 40 pr 5-6-day-old B. longicaudatus adults which had had no prior oviposition experience. Oviposition units each containing ca. 55 larvae of a given age were introduced into each cage. Larvae 1-7-days-old[1] were provided and each age group was represented in 3 oviposition units resulting in a total of 21 units/cage. Oviposition units were arranged as in Fig. 4. Entry into the cage was via the "sleeve end" and parasitoid activity was observed through the "glass end" (Fig. 4). After the 24 hr exposure period, oviposition units were removed from the cages and larvae which were preparing to pupate were put in containers with moist vermiculite. Younger larvae were put in fresh bagasse diet until ready for pupation. Parasitoid progeny were counted and expressed as a percentage of the number of puparia produced for each of the seven host ages.

Results and Discussion

Five-day-old larvae produced significantly ($p < 0.05$) more

[1]Seven-day-old larvae were the largest and most mature available before pupation.

Figure 4. Arrangement in cage, of oviposition units containing 1-7-day-old Anastrepha suspensa larvae.

Numbers indicate age of larvae in oviposition units within the cage.

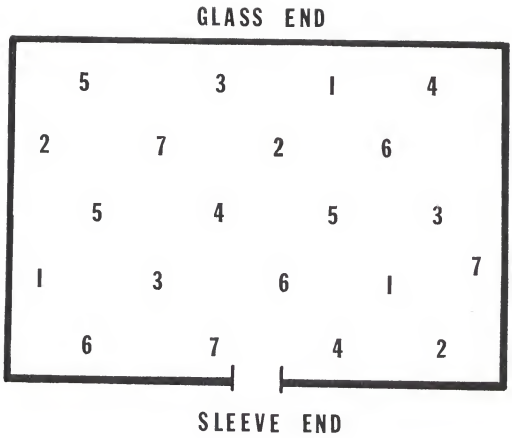

GLASS END
5 3 1 4
2 7 2 6
5 4 5 3
1 3 6 1 7
6 7 4 2
SLEEVE END

Table 4.--Percent emergence of Biosteres longicaudatus progeny from 1-7-day-old Anastrepha suspensa larvae

Replicate*	% parasitoid emergence from A. suspensa larvae of given ages (days)						
	1	2	3	4	5	6	7
A	0.8	13.5	3.3	10.3	47.3	12.6	16.7
B	0.0	1.5	15.5	5.1	68.6	18.8	4.8
C	0.0	0.9	9.2	13.3	47.1	30.9	17.9
D	0.0	0.0	7.1	2.6	8.6	11.9	7.1
E	0.0	2.1	7.7	2.4	42.1	13.3	4.9
Total :	0.8	18.0	42.8	33.7	213.7	87.5	51.4
Mean** :	0.16^{a}	3.60^{b}	8.56^{cf}	6.74^{c}	42.74^{d}	17.5^{e}	10.28^{f}

*Each replicate consisted of 40 pr 5-6-day-old B. longicaudatus adults and 21 oviposition units each containing larvae 1-7 days old.

**Numbers followed by the same letter are not significantly different by the Duncan's new multiple range test at the 0.05 level.

parasitoid progeny than larvae of other ages (Table 4). If we consider suitability not only as a measure of quality but also of quantity, then it could be concluded that 5-day-old hosts were quantitatively suitable for parasitoid development. The very low parasitoid emergence rates observed for 1-4-day-old hosts could have been a result of the small size of larvae of those ages, or a combination of size and other factors. One would have expected an increase in number of progeny with an increase in size of hosts if host size was the only factor affecting parasitoid emergence. However, the fact that mean percent parasitization (=number of parasitoids divided by total number of puparia recovered x 100%) in 7-day-old hosts (the largest larvae) was not significantly different from that of 3-day-old hosts (Table 4), is an indication that other factors in addition to size were involved.

Host Age Preference of B. longicaudatus Females as Indicated by Egg Distribution

Materials and Methods

An experiment was designed to determine whether B. longicaudatus females preferentially oviposited in host larvae of a specific age and thus caused the discrepancies in parasitoid survival observed in Table 4. Two oviposition units were exposed simultaneously to 20 5-6-day-old B. longicaudatus females for 24 hr. One unit contained 100 5-day-old host larvae and the other, 100 7-day-old larvae. This was replicated 5 times. After parasitization, larvae were left for an additional 24 hr to allow for enlargement of parasitoid eggs. Twenty-five specimens were removed from each age group and preserved in 70% EtOH for dissection. The number of eggs found was noted and the data were analyzed by analysis of variance.

Results and Discussion

The numbers of parasitoid eggs found in 7 and 5-day-old larvae were not significantly different (Table 5). Only larvae of these 2 ages were used for this experiment because 5-day-old larvae, though smaller, gave significantly more parasitoids than 7-day-old hosts. Since the data in Table 5 showed no ovipositional preference for larvae regardless of size, the reasons for differences in parasitoid emergence appear to be within the host larvae themselves. Thus subsequent experiments were conducted to determine what other effects hosts of different ages may have on parasitoids. This information would be invaluable in a mass-rearing program as Salt (1938, 1941), Arthur and Wylie (1959), Lewis and Redlinger (1969) and other workers have demonstrated that hosts can affect their parasitoids.

Effect of Host Age on Development of B. longicaudatus

Materials and Methods

Three aspects of the parasitoid's development were investigated. These were: the effect of host age on the duration of parasitoid immature stages, the effect of the hosts on the size of parasitoid progeny, and the percent survival of immature parasitoids in hosts of different ages. Oviposition units were prepared as described under "Rearing of B. longicaudatus." A. suspensa larvae aged 4-7 days were used. About 200-250 larvae of the same age and approximately the same size were placed in each of 2 oviposition units with 8 g bagasse. Larval age was confirmed using mouth hook morphology (Fig. 1). Larvae were exposed for 24 hr to 40 pr of

Table 5.--Egg distribution by female Biosteres longicaudatus in 5 and 7-day-old Anastrepha suspensa larvae

Replicates*	No. eggs / 25 A. suspensa larvae		
	5-day-old	7-day-old	Total
A	55	53	108
B	49	51	100
C	53	56	109
D	44	47	91
E	37	40	77
Total	238	247	485
Mean	47.6^{ns}	49.4^{ns}	97.0

*Each replicate consisted of 20 5-6-day-old B. longicaudatus females and 100 each, of 5 and 7-day-old larvae.

^{ns}Not significantly different by the analysis of variance at the 0.05 level.

5-6-day-old B. longicaudatus adults. Parasitized larvae were allowed to pupate in containers of moist vermiculite where they remained until parasitoid emergence. Twenty to 40 larvae or pupae were removed from each age group of parasitized larvae at regular intervals until parasitoid emergence. Specimens were placed in 70% EtOH for subsequent dissection.

Serial dissection of parasitized hosts facilitated a determination of the duration and percent survival of each parasitoid immature stage. Percent survival of each immature stage was obtained by expressing the number of each stage found as a percentage of the number of parasitized hosts dissected. Adult parasitoids were counted and then measured lengthwise from the vertex of the head, along the dorsum to the tip of the abdomen, exclusive of the ovipositor.

Results and Discussion

Investigation of the duration of the egg stage of the parasitoid within 4-7-day-old hosts revealed that in 6 and 7-day-old hosts, the egg stage lasted significantly longer ($p < 0.05$) than in 4 and 5-day-old hosts (Table 6). There appeared to have been little egg development (as evidenced by lack of egg enlargement), as late as 3 and 5 days after oviposition in 7-day-old larvae whereas eggs in preferred hosts hatched in ca. 2 days. Eggs in 7-day-old hosts often retained the sickle shape common to newly oviposited eggs and some were white and hardened. In many cases, 7-day-old larvae were rotten and eggs which were found were either slightly brown and transparent or collapsed. It is not known whether the above mentioned white eggs found up to 5 days after oviposition would have hatched eventually. However, these did not look like healthy eggs which are translucent,

Table 6.--Influence of age of Anastrepha suspensa larvae on developmental time of its parasitoid, Biosteres longicaudatus

Age of host larvae (days)	Mean duration (days) of parasitoid immature stages* ++					Total parasitoid developmental time (days)* ++		
	Parasitoid larval stages+							
	Egg**	1	2	3	4	Pupa	Female	Male
4	(18) 2.0[a]	(15) 2.0	(12) 2.0	(12) 2.0	(11) 3.8	(12) 6.5[a]	(10) 19.0[a]	(15) 18.0[a]
5	(18) 2.0[a]	(20) 2.0	(16) 2.0	(14) 2.0	(11) 4.0	(10) 6.4[a]	(28) 19.0[a]	(25) 18.0[a]
6	(20) 2.7[b]	(15) 2.0	(10) 2.0	(10) 2.0	(10) 4.1	(14) 6.7[a]	(15) 20.0[b]	(10) 19.0[b]
7	(18) 4.6[c]	(10) 2.1	(12) 2.0	(10) 2.2	(6) 4.1	(10) 7.5[b]	(9) 23.0[c]	(5) 22.0[c]

* Numbers in the same column followed by the same letter are not significantly different ($p<0.05$) by the Duncan's new multiple range test.

** The 24 hr period during which oviposition occurred is not included in these figures.

\+ Analysis of variance showed no significant differences ($p<0.05$) in duration of larval stages.

++ Numbers in parentheses in each column indicate sample size.

with a clear serosal area. The pupal stage was significantly prolonged ($p < 0.05$) in 7-day-old hosts also. Larvae that were dissected showed a high percent of rotting, indicated by dark brown tissue. There was no apparent effect of host age on larval duration in those cases in which parasitoid eggs did hatch. The fact that only egg and pupal stages were affected is a matter that needs further investigation. There were no similar observations in any of the other hosts. It is possible that the extreme results observed for parasitoids reared in 7-day-old hosts may be due to the physiological condition of the hosts but this matter will be discussed later in the dissertation.

Surprisingly, 6 and 7-day-old hosts produced parasitoids that were significantly larger ($p < 0.05$) than those from other hosts (Table 7, Fig. 5). The use of large individuals may have some advantage in mass rearing programs, but the fact that 7-day-old hosts retard the development of parasitoids, is a factor to consider before choosing these larvae for mass rearing the parasitoid.

Survival of all parasitoid stages was lowest in 7-day-old hosts and highest in 5-day-old hosts (Fig. 6). As a result very few individuals were available from these hosts for observation (Table 6). The parasitoids took the longest time to develop from egg to adulthood in 7-day-old larvae (Table 6) and this was possibly due to the retarded egg and pupal developments. The high mortality (ca. 50%) of hosts observed in dissections could have been the reason for the low percent survival observed in Fig. 6 for both male and female parasitoids. It is possible that the physiological condition of the hosts at the time of parasitization determined the degree of success in the development of parasitoid progeny.

Table 7.--Influence of age of Anastrepha suspensa larvae on size of adult Biosteres longicaudatus

Age of larvae (days)	Size (mm) of adult parasitoid progeny*					
	Female**			Male**		
	n	Range	Mean	n	Range	Mean
4	10	3.5 - 3.8	3.50[a]	10	2.5	2.50[a]
5	20	3.5 - 3.8	3.65[b]	20	2.5	2.50[a]
6	15	3.8	3.80[c]	10	2.5 - 2.8	2.62[b]
7	9	3.5 - 4.0	3.85[c]	5	2.8	2.80[c]

*Adults were measured lengthwise from the vertex of the head to the tip of the abdomen, exclusive of the ovipositor.

**Numbers in the same column followed by the same letters are not significantly different at 0.05 level by Duncan's new multiple range test.

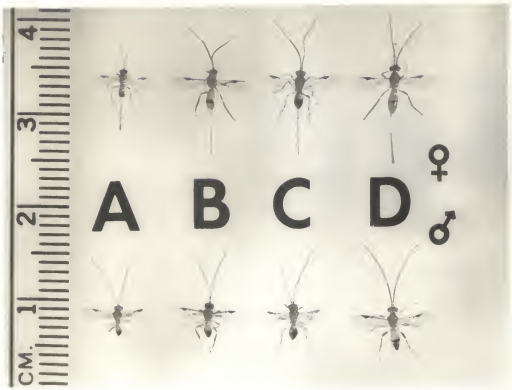
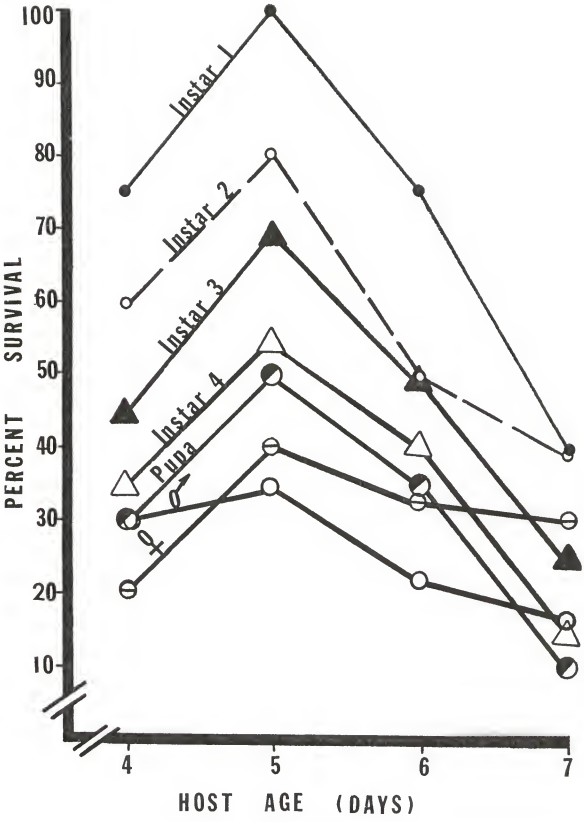


Figure 5. Male and female Biosteres longicaudatus which emerged from 4-7-day-old hosts.

A, B, C, D emerged from 4, 5, 6 and 7-day-old hosts, respectively.

Figure 6. Effect of host age on percent survival of developmental stages of Biosteres longicaudatus.


100
90
80
70
60
50
40
30
20
10
PERCENT SURVIVAL
Instar 1
Instar 2
Instar 3
Instar 4
Pupa
♂
♀
4
5
6
7
HOST AGE (DAYS)

Determination of the Critical Period in *A. suspensa* Larvae

Materials and Methods

An effort was made to associate the period of optimal host age suitability to changes in the host larvae. The most obvious change, pupation, was suspected to be associated with changes in host suitability. For normal holometabolous insects, the critical period may be described as that period during which the brain hormone is required to stimulate the prothoracic glands (which are part of Weismann's gland in Diptera) to produce sufficient ecdysone that will initiate pupation (Chapman 1969). Newly molted 3rd instar *A. suspensa* larvae were dissected and the location of the ring gland noted. Intact larvae were ligated with surgical thread posterior to the ring gland. Larvae were ligated on the 1st and 2nd days of the 3rd instar (5 and 6 days old, respectively). Fourteen to 18 larvae were ligated and these were replicated 3 times each day ligations were performed. Ligated larvae were held in petri dishes on moist filter paper. They were observed at least twice per day to determine how many had pupated. Ideally, larvae ligated posterior to the ring gland before the critical period should pupate anteriorly only. The posterior end should remain larviform. Larvae ligated posterior to the ring gland after the critical period should pupate both anterior and posterior to the ring gland (De Bach 1939). Using these criteria, the critical period of *A. suspensa* larvae was estimated. Ligated larvae were compared with unligated controls regarding the time of pupation.

Results and Discussion

Because other workers (Cals-Usciati 1969, Smilowitz 1974) have implicated hormones, specifically the molting hormone, as being important to suitability of hosts for parasitoid development, I was prompted to investigate the same matter with respect to B. longicaudatus. The differential survival of parasitoids in hosts of different ages (Table 4) and the effects of host ages on the duration of parasitoid development were the motivating forces behind an investigation of endocrine involvement in host suitability. A mean of 81.3% of healthy A. suspensa larvae pupated between the 7th and 8th days (Table 8). It should be noted that most of the pupation occurred late on the 7th day. Pupation in entirety by 2.1% of the larvae ligated on the 5th day (Table 8) could be an indication of imperfect ligation technique. Some ecdysone might have entered the posterior end of the larvae if the ligatures were not tight enough. The occurrence of 58.4% pupation anteriorly and only 12.5% posteriorly and entirely, in larvae ligated on the 5th day, is an indication that the majority of larvae had not yet passed the critical stage on the 5th day of larval development. The fact that in some cases pupation occurred in posterior regions only, may be attributable to the ligatures not being far enough posterior to the ring gland, hence hormone might have been secreted into this area. The occurrence of 56.4% pupation posterior to ligatures and in entire larvae of those ligated on the 6th day, is an indication that the critical period had already passed in these cases. Ligated larvae suffered high mortality due to disease organisms which caused blackening especially in posterior areas.

Table 8.--Determination of the critical period in Anastrepha suspensa by ligation of 5 and 6-day-old larvae

Location of pupation*	No. of larvae pupating in hr.+ 12	24	48	72	No. larvae not pupated	Total	
	--------Ligated on 5th day**-----						
A	---	2(4.2)	26(54.2)	--	---	28	
P	---	---	---	1(2.1)	---	1	48
T	---	1(2.1)	4(8.3)	--	14	19	
	-------Unligated control---------						
A	---	---	---	--	---	---	
P	---	---	---	--	---	---	
T	---	5(10.4)	39(81.3)	4(8.3)	---	48	
	-------Ligated on 6th day**-----						
A	8(16.6)	1(2.1)	---	---	---	9	
P	2(4.2)	3(6.3)	---	---	---	5	48
T	9(18.8)	13(27.1)	---	---	12	34	
	-------Unligated control--------						
A	---	---	---	---	---	---	
P	---	---	---	---	---	---	
T	11(22.9)	32(66.7)	3(6.2)	2(4.2)	---	48	

+Numbers in parentheses represent percent pupating.

*A, P, T = Pupation anterior to ligature, posterior to ligature, and total pupation, respectively.

**Ligations made posterior to the ring gland.

Table 9.--Percent emergence of Biosteres longicaudatus progeny from hosts which were parasitized before (early 3 instar) or after (late 3rd instar) the critical period

	Parasitoid emergence from hosts of given ages at time of parasitization*			
	%		♀ : ♂ ratio	
Host age:	5	7	5	7
Replicate**				
A	72.2 (187)	31.2 (105)	1.0:1.13	2.60:1.0
B	62.7 (207)	26.4 (99)	1.0:1.38	1.75:1.0
C	60.5 (130)	44.1 (75)	1.0:3.10	2.75:1.0
Total	195.4 (524)	101.7 (279)	3.0:5.61	7.10:3.0
Mean	65.1 (174)	33.9 (93)	1.0:1.87	2.37:1.0

*Numbers in parentheses refer to number of puparia in sample.

**Each replicate consisted of 24 pr 5-6-day-old B. longicaudatus adults and 700 5 or 7-day-old A. suspensa larvae.

Hence, there is a possibility that any tanning due to pupation might have been obscured. From the data in Table 8 there is some indication that 5-day-old larvae had not yet reached the critical period while 7-day-old larvae had exceeded the critical period. It was established earlier using mouth hook size and morphology (Table 1, Fig. 2) that 5 and 7-day-old larvae were in the early and late stages, respectively, of the 3rd instar.

The determination of the critical period is an indication that processes, possibly hormonal, associated with the onset of pupation in 7-day-old (late 3rd instar) larvae, may have been the cause of decreased emergence of parasitoid progeny, and prolonged duration of egg and pupal stages of the parasitoid. Table 9 shows the percent emergence of parasitoids from 5 and 7-day-old hosts after the critical period was established. The percent of offspring from 5-day-old hosts was still higher than those from 7-day-old hosts. A similar relationship was observed in Table 4, though the proportions were somewhat higher. The duration of parasitoid egg development showed a significant increase in post-critical period (7-day-old) larvae compared with younger hosts. This finding reinforced the data given in Table 6. Table 9 also shows that there is a higher ratio of female to male parasitoids in older hosts than in younger hosts.

Use of a Juvenile Hormone Analogue to Prolong the Larval Stage of A. suspensa

Materials and Methods

The fact that ecdysone and juvenile hormone occur in varying titers during an instar (Patel and Madhaven 1969) reinforced the suspicion that either one or both of these hormones might have

contributed to the partial unsuitability observed in the late 3rd instar. Thus Altozar 53%, a juvenile hormone analogue (JHA) produced by the Zoecon Chemical Compnay, Palo Alto, California, was applied to larvae in an effort to increase the juvenile hormone titer in the hemolymph of 7-day-old larvae and simulate the pre-critical period condition. Two techniques were used for JHA application: injection and topical application. Five concentrations of JHA were used. The JHA was dissolved in acetone for topical treatments, and in Planters(R) peanut oil for injection. Two microliters of various concentrations of JHA were applied (topically or injected) to each larva. The mean weight of 20 larvae facilitated calculation of the dosage of actual JHA applied per gram of mean larval body weight. Dosages of actual JHA applied per gram of mean larval weight were:

0.45 µg/g
0.95 µg/g
1.80 µg/g
2.77 µg/g
3.70 µg/g

Of the above mentioned dosages, 2.77 µg/g was chosen for use in further experiments. This dosage prolonged the larval stage and resulting pupae suffered minimal dessication within the 1st 8 days of treatment.

Larvae were topically treated or injected with 2 µl of 2.77 µg/g actual JHA during their 1st and 2nd days in the 3rd instar. The second treatment was administered within 12-15 hr of the first. All injections were made beneath the integument in the lateral, lower 2/3rd of the body. The 37 gauge hypodermic needle used was attached to a syringe which was driven by a repeating dispenser that regulated the microliter amounts applied to the larvae. Controls were injected or topically treated with peanut

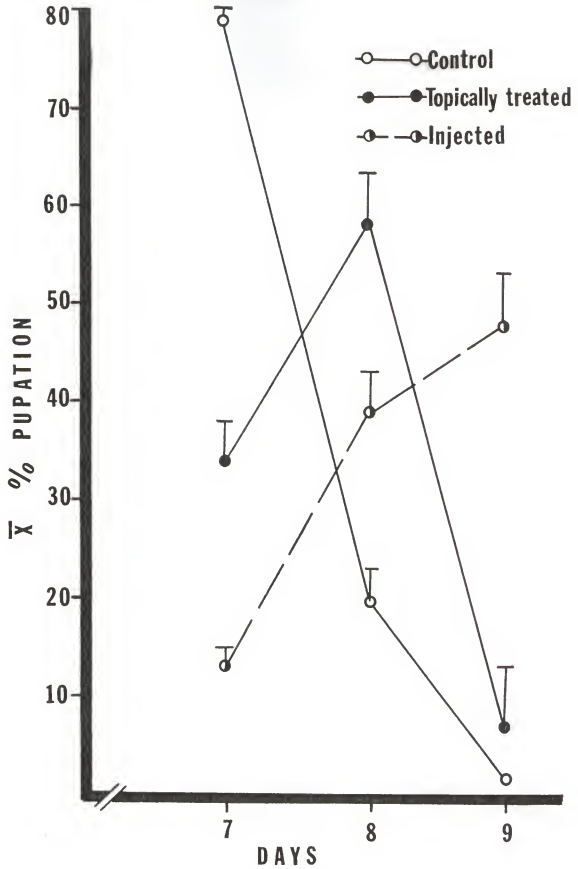
oil or acetone, respectively. These carriers did not themselves affect development. All larvae were anaesthetized for 2 min with CO_2 prior to treatment. Between 130 and 190 larvae were treated in each of the 3 replicates used. From each replicate, 50-80 treated larvae were removed for observation of time of pupation. The remainder were exposed to parasitoids for investigations on host suitability. The pupation time of treated larvae was compared to those of untreated controls to determine the effect of JHA on the physiological condition and hence duration of the larval stage.

Results and Discussion

The treatment of larvae with JHA was done primarily to change the juvenile hormone:ecdysone balance if possible, in an effort to reinstate in post-critical period larvae, the pre-critical period condition observed in 5-day-old larvae. It has been established by Wigglesworth (1954), Williams (1956) and others that in the species studied, juvenile hormone titer is higher during younger stages of larvae than in more mature larvae. Thus it was hoped that injection of JHA would have a juvenilizing effect by increasing JHA titer in the larval hemolymph. This situation, if achieved, might result in an increase in the period that larvae could be suitable to parasitoids. The fact that certain enzymes are known to inactivate JH at strategic ontogenetic points (Gilbert and Schneiderman 1958), was taken into account and an effort was made to keep a high enough amount of JHA in the hemolymph to compensate for any enzymatic inactivation.

The highest percent of pupation on the 7th day was in the untreated controls (Fig. 7). Pupation times of the highest percents

Figure 7. Delay in time of pupation of Anastrepha suspensa larvae due to treatment with juvenile hormone analogue (JHA) at a dosage of 2.77μg JHA per gram of mean larval weight.


80
70
60
50
40
30
20
10
X̄ % PUPATION
Control
Topically treated
Injected
7
8
9
DAYS

A. B. C.

Figure 8. Effect of juvenile hormone analogue (JHA) treatment of Anastrepha suspensa larvae on their resulting pupae

A. Injected with JHA (2.77µg actual per gram of mean larval weight)

B. Topically treated with JHA (2.77µg actual per gram of mean larval weight)

C. Untreated control

of topically treated and injected larvae were on the 8th and 9th days, respectively. Thus treatment of larvae with JHA substantially prolonged the larval stage and resulted in late pupation. A larviform condition persisted in pupae resulting from JHA injected larvae (Fig. 8A) and there was some deformation of these from topically treated larvae (Fig. 8B), compared with pupae from untreated controls (Fig. 8C). The deformation observed is in agreement with the observations of other workers on several insect species (Bull, Ridgway, Buxkemper, Schwarz, McGovern, and Sarmiento 1973; and Neal, Bickley, and Blickenstaff 1970).

Effect of Juvenile Hormone Analogue on Host Suitability

Materials and Methods

Treated larvae on the 3rd day of the 3rd instar (7 days old) were exposed for 24 hr to 5-6-day-old B. longicaudatus females in a host:parasitoid ratio of about 30:1. Untreated 5 and 7-day-old larvae were similarly parasitized. After parasitization, samples were removed from parasitized larvae and preserved in 70% EtOH for dissection. This was done in order to determine the duration of the parasitoid egg stage. A comparison of the duration of the egg stage would indicate any change in egg duration in larvae treated with JHA. The remaining parasitized larvae were allowed to continue development until parasitoids emerged. Comparison of the number of parasitoids emerging from JHA treated, and 5 and 7-day-old untreated larvae would indicate any change in suitability of treated larvae, which were parasitized at a time when they would normally have been unsuitable; i.e., 7 days old.

Results and Discussion

Earlier observations showed that the duration of the egg stage was prolonged significantly in 7-day-old hosts (Table 6) and that percent parasitoid emergence was considerable lower than in 5-day-old larvae (Table 9). Hence an investigation of the effect of JHA on suitability was made with respect to duration of egg stage and percent parasitoid emergence. Only these 2 aspects of suitability were investigated because no other signs of unsuitability were observed. Encapsulation of parasitoids by the host was not investigated because there were no observations which showed a predominance of encapsulation by hosts of any age. The only forms of encapsulation observed were in the case of conspecific, parasitoid lst instars killed by fighting among themselves.

The mean duration of the egg stage of the parasitoid was not prolonged in JHA treated or untreated control larvae but was prolonged significantly ($p < 0.05$) in 7-day-old untreated controls (Table 10). As both the JHA treated and the untreated 7-day-old larvae were parasitized on the 7th day, differences observed in egg duration were presumably due, not to the chronological age of larvae at time of parasitization, but to their physiological state. In the case of JHA treated larvae, the physiological state was evidently altered. Thus larvae which were normally unsuitable (7-day-old) for parasitoid egg development were made more suitable with JHA.

Further work needs to be done before the effect of JHA on egg duration can be confirmed. In in vivo situations such as the one in which these experiments were carried out, it is difficult to come to conclusions as to the direct effect of the hormone analogue

Table 10.-- Duration of the egg stage of Biosteres longicaudatus in hosts treated with a juvenile hormone analogue (JHA) at a rate of 2.77µg actual JHA per gram of mean host tissue

Replicate**	No. eggs hatching * in n days 2	3	4	5	6	Total no. eggs	$\bar{x}$ egg duration+
	Topical treatment++						
A	10	--	--	--	--		
B	8	--	--	--	--		
C	6	--	--	--	--		
Total	24	--	--	--	--	24	2.0[a]
	Injected treatment++						
A	7	--	--	--	--		
B	9	1	--	--	--		
C	9	--	--	--	--		
Total	25	1	--	--	--	26	2.03[a]
	Untreated 5-day-old						
A	11	1	--	--	--		
B	13	--	--	--	--		
C	9	--	--	--	--		
Total	33	1	--	--	--	34	2.02[a]
	Untreated 7-day-old						
A	--	1	--	9	1		
B	1	7	6	--	--		
C	--	1	5	3	--		
Total	1	9	11	12	1	34	4.08[b]

+Numbers in the same column followed by the same letter are not significantly different from each other at the 0.05 level by Duncan's new multiple range test.

++2.77µg actual juvenile hormone analogue applied per gram of mean larval body weight.

*Based on appearance of 1st instars in samples taken 0-8 days after parasitization.

**Each replicate consisted of 35-50 parasitized larvae taken at random from 3 groups of larvae exposed to parasitoids at ca. 30:1 ratio.

because many other factors are associated with the internal host environment. The most ideal method that could be used in ascertaining JHA effects would be by in vitro study. In this case, the dosage of JHA could be varied and the qualitative and quantitative effects could be measured. In vitro studies could be invaluable in defining the optimal environmental conditions for parasitoid egg development. JHA injected larvae gave considerably fewer parasitoids compared with other experimental and control hosts (Table 11). This low yield was possibly due to the trauma of 2 injections within 12-15 hr of each other. When parasitoid emergence from topically treated larvae is compared with that from other hosts, it can be seen that topical treatment of larvae appeared to improve the suitability of larvae somewhat, compared to untreated 7-day-old larvae. However, untreated 5-day-old larvae were still the most suitable hosts of all those used in the experiment (Table 11).

Since the host:parasitoid ratio was held at approximately 30:1 in the experiment, differential egg deposition by parasitoids can be ruled out as an explanation for the differential numbers of parasitoids produced in the various groups. During dissection for eggs, no wide variation in egg distribution was observed, so it is unlikely that injected larvae received so many more eggs than other hosts that such high mortality occurred.

As for the egg stage, in vitro studies could be used to shed light on specific involvements of the host endocrines with parasitoid development. Some workers reported that application of JHA on hosts did not affect parasitoid development; e.g., Wilkinson and Ignoffo (1973) reported that topical dosage of a juvenile hormone analogue

Table 11.--Effect of a juvenile hormone analogue (JHA) on percent emergence of Biosteres longicaudatus progeny from hosts treated with 2.77µg actual JHA per gram of mean host tissue

	% parasitoid emergence[+]				
Replicates* :	A	B	C	Total	Mean**
		Topical treatment			
	63.6 (28)	55.8 (53)	47.7 (41)	167.1	55.7[a]
		Injection			
	20.0 (21)	37.8 (36)	17.3 (17)	75.1	25.0[b]
		Untreated 5-day-old larvae			
	66.0 (63)	90.7 (88)	82.0 (67)	238.7	79.5[c]
		Untreated 7-day-old larvae			
	40.6 (61)	51.6 (47)	35.6 (31)	127.8	42.6[d]

[+]Numbers in parentheses represent sample size.

*Each replicate consisted of larvae exposed to parasitoids in a ratio of ca. 30:1.

**Numbers in the same column followed by the same letter are not significantly different at the 0.05 level by the Duncan's new multiple range test.

on Pieris rapae (L.) affected neither emergence, longevity nor sex ratio of Apanteles rubecula Marshall. They found that 73% parasitoid emergence was obtained with topical applications. Wright and Spates (1972) also reported no effect of JHA on Muscidifurax raptor Girault and Sanders which emerged from treated pupae of the stable fly. Other workers however, have shown ill effect of JHA on parasitoids. Vinson (1974) reported delayed and reduced emergence of Cardiochiles nigriceps adults from Heliothis virescens treated with a JH analogue, and McNeil (1975) observed that pupae and larvae of Aphidius nigriceps died when its host, Macrosiphum euphorbiae was treated with a juvenile hormone analogue. The conflicting arguments concerning JHA effects on beneficials need to be elucidated through refined experimentation. The juvenile hormone analogues produced by chemical companies vary in their chemical structures although the active moieties are similar and this could be one reason for the varying reports on JHA effects. It is important for biological control programs, which employ beneficial insects as control agents, that the role of juvenile hormone analogues be investigated more thoroughly.

CHAPTER V

REPRODUCTIVE BIOLOGY OF B. LONGICAUDATUS

Introduction

There is little information on the reproductive potential of B. longicaudatus. Initial investigation of the reproductive capabilities of this parasitoid might help improve mass rearing techniques. Greany et al. (1976) reported that females of B. longicaudatus have no obligatory premating period. This fact was reconfirmed in the present study. B. longicaudatus (Fig. 9) is arrhenotokous, in that unfertilized eggs produce male offspring. Observations in the laboratory indicated that the ratio of female to male offspring increased with the age of the host parasitized (Lawrence, Baranowski, and Greany 1976).

The reproductive capacity of some parasitoids was found to be affected by temperature (Ragusa 1974) as well as by the frequency of exposure to hosts; e.g., females of Microtonus aethiops laid more eggs when exposed to adult Hypera postica every 24 hr than when they were offered hosts every 2 or 3 days (Fusco and Hower 1974). These workers also found that more female progeny resulted when oviposition was delayed due to lack of hosts and 26°C was the optimum for egg production and longevity of Microtonus females.

In the investigation of B. longicaudatus reproductive biology, an effort was made to determine whether females were 'synovigenic' or 'proovigenic', and the extent to which frequency of oviposition

Figure 9. Female of Biosteres longicaudatus ovipositing in guava fruit infested with larvae of Anastrepha suspensa.

experience affected the rate at which females produced mature eggs. Female insects which emerge with a complete complement of mature eggs, deposit them in a short time and develop no more eggs, are said to be 'proovigenic' (Flanders 1950). Conversely, 'synovigenic' females continue to produce mature eggs throughout their adult life (Flanders 1950). As a consequence the quality of eggs produced by 'synovigenic' females is determined by their nutrition.

Relationship Between Age of B. longicaudatus Females and Number of Eggs in Their Ovaries in the Absence of Hosts

Materials and Methods

A large number of B. longicaudatus adults, all of which had emerged within a 24 hr period, were collected and fed honey and water in the usual manner. Adults were held in plexiglas cages at about 26°C and 60% RH. No hosts were provided during the holding period. Samples of 25-30 females were removed from the cages every other day for 15 days, and preserved in ethanol. The number of mature eggs within the ovaries of females of each age was recorded. Eggs were considered to be mature if they were turgid and sickle shaped with a clear serosal area. Most of these were stored in the lower portion of the lateral oviducts. All females dissected were assumed to be mated as equivalent numbers of males and females were held together, and mating was observed among individuals less than 24 hr old. No virgin females were used in this experiment.

Results and Discussion

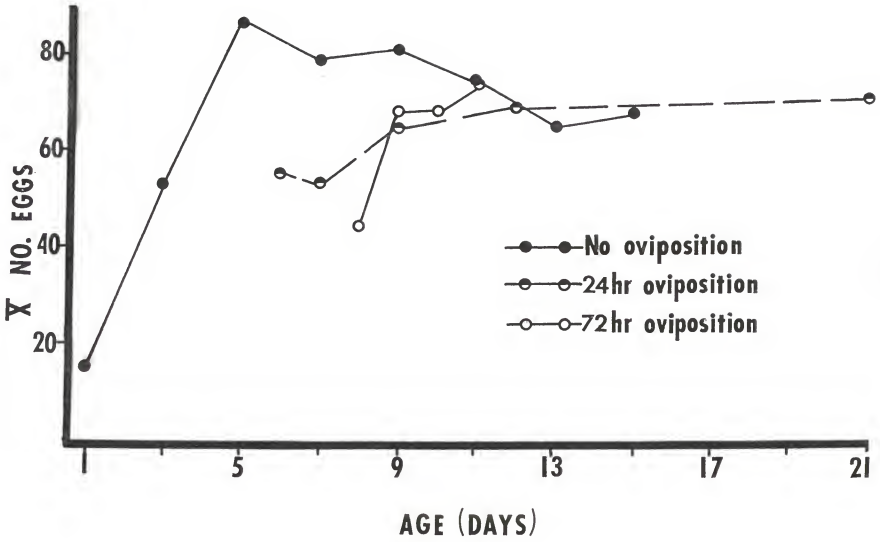
Fig. 10 shows an increase in the number of mature eggs in ovaries of mated B. longicaudatus females with increase in age

up to 5 days. There was a slight but perhaps insignificant decrease in the number of mature eggs as females continued to age, indicating that some egg resorption had occurred (Fig. 10). Doutt (1964) indicated that some parasitoids resorb their eggs when they are deprived of hosts. As heavy parasitoid mortality occurred when parasitoids were 16 days old, further decreases in the number of mature eggs could not be determined. It is noteworthy that a relatively large number of mature eggs was maintained in ovaries throughout 3/4 of the adult life, indicating high biotic potential. Greany et al. (1976) also noted this phenomenon and commented on the need to develop a means of stimulating females to deposit a majority of these eggs in order to increase parasitoid production.

Greany et al. (1976) found a disparity between the number of mature eggs within B. longicaudatus ovaries and the actual rate of progeny production. They wondered whether their observation that 20-30 progeny were produced in a female's lifetime was due to low rates of oviposition. During studies on host discrimination involving dissection of hosts and counting of parasitoid eggs, I found that 20-30 eggs were laid by a 5-day-old female in a day providing adequate numbers of hosts were available. This indicated that low progeny rates observed by Greany et al. (1976) were probably due to either host unsuitability or too few hosts were exposed to parasitoids.

Fig. 10 shows that 5-day-old females had the highest egg laying potential. Five days would seem to be the best age at which female parasitoids should first be exposed to hosts since I have observed that more male progeny were produced when younger females were used.

Figure 10. Mean number of mature eggs within ovaries of mated Biosteres longicaudatus females of different ages.


80
60
40
20
X NO. EGGS
No oviposition
24hr oviposition
72hr oviposition
1
5
9
13
17
21
AGE (DAYS)

Determination of 'Proovigenic' or 'Synovigenic' Condition of B. longicaudatus Females

Materials and Methods

The fact that mated females of B. longicaudatus mature eggs as they age is an indication of the 'synovigenic' condition described by Flanders (1950). The females observed in the preceding study on reproductive biology were not provided with hosts however, so it was not known whether they were able to replenish their egg supply after oviposition. To investigate this, I exposed 5-day-old A. suspensa larvae to parasitoid females and their male partners of similar age, in a host:female parasitoid ratio of ca. 30:1. Oviposition lasted 24 hr. Fifteen to 20 of these parasitoid females were preserved in ethanol immediately after oviposition (0 days) and 1, 3, 6 and 15 days later, when females were 6, 7, 9, 12 and 21 days old, respectively. The number of mature eggs within the ovaries of females provided with hosts for 24 hr, was compared with the number in females of equivalent ages which had not had any oviposition experience. The data from females with oviposition experience were also used to determine the effect of a single oviposition experience on the rate at which females matured their eggs.

A second experiment was conducted to determine the effect of 72 hr continuous oviposition experience on the rate at which females matured their eggs. The parasitoids and hosts used were 5 days old (hosts were replaced every 24 hr) and the same host:parasitoid ratio as used for the 24 hr oviposition experiment was maintained.

Fifteen to 20 females were preserved in alcohol immediately after the oviposition period, when parasitoids were 8 days old, and each subsequent day for 3 days. The number of mature eggs in ovaries of these females was compared with that of females with a single oviposition experience.

Results and Discussion

Even after oviposition, B. longicaudatus females were found to be able to mature more eggs (Fig. 10). As a result, they possessed about the same number recorded for females of corresponding ages which had not oviposited. Thus B. longicaudatus females are 'synovigenic'. This observation is in agreement with that of Greany et al. (1976).

Females that were provided with larvae for 72 hr had a mean of 44 mature eggs at the end of the oviposition period when they were 8 days old (Fig. 10). One day later, a mean of 68 eggs was observed. Two days later, the mean number of eggs was increased to 75.5. Thus females with 72 hr oviposition experience increased their mean number of eggs by 31.5 in 3 days (Fig. 10). However, females with only 24 hr oviposition experience had increased their mean number of eggs by a mere 14.1 in 6 days (Fig. 10). It therefore appears that frequent oviposition stimulated females to mature their eggs at an accelerated rate. Similar results were obtained by Fusco and Hower (1974) with Microtonus aethiops, a parasitoid of the alfalfa weevil. Further investigations are necessary to determine the effect of continuous exposure of hosts to parasitoids on the rate at which females mature their eggs. This phenomenon could also affect

the rate of oviposition.

Since females are 'synovigenic' and since the latter condition is related to the parasitoid's nutrition, further investigations could be done in an attempt to develop a diet that would help to improve the number and quality of parasitoid offspring. If any such findings were implemented in conjunction with frequent exposure of hosts to parasitoids, the end result could be of significance in a mass rearing of large numbers of viable parasitoids.

CHAPTER VI

SUMMARY AND CONCLUSION

The Caribbean fruit fly, Anastrepha suspensa (Loew) was reared in sugar cane bagasse diet at high humidity and 26°C. There are 3 instars, with characteristic mouth hooks 40.0μ, 145.0μ and 215.0μ long, respectively. The period from egg to fly emergence was 19-21 days. Biosteres (=Opius)longicaudatus, a solitary endoparasitoid was reared on 5-day-old A. suspensa larvae which were found to be optimally suitable for attack and development. The duration of the life cycle of B. longicaudatus from egg to adult emergence was 18-22 days for males and 19-23 days for females at ca. 26°C and 60% RH. The proportion of male to female progeny produced was related to the age of the host at the time of exposure, with relatively more females resulting from older hosts. This discrepancy might have been due to differential survival of the sexes or by regulation of fertilization by parent B. longicaudatus females at the time of oviposition.

Host:parasitoid ratios of 6.3:1, 14.6:1 and 23.3:1 were used for studies on the host discriminating capability of B. longicaudatus females. The ability to distinguish parasitized hosts was evident within 24 hr of initial parasitization at the 2 higher host:parasitoid ratios. Superparasitization was highest at the lowest host: parasitoid ratio and this was concomitant with increased mortality and low parasitoid progeny emergence. At 6.3:1 and 14.6:1 ratios, female parasitoids restrained themselves and each laid fewer eggs.

More eggs were laid per female at the highest host:parasitoid ratio of 23.3:1, but the number of parasitoid progeny which emerged was not significantly higher than at the 14.6:1 ratio. In the former case many hosts received no eggs, hence many larvae escaped parasitization. The 14.6:1 ratio therefore appeared to be the ratio most economical for mass rearing.

Parasitoids were reared on 4-7-day-old hosts, and the duration of each developmental stage was observed in each host age. The number of parasitoids emerging was also noted. The highest percentage of parasitoid emergence occurred from 5-day-old hosts. Significantly prolonged egg and pupal stages of the parasitoid were noted in 7-day-old hosts and significantly fewer parasitoids also emerged from those hosts.

The possible influence of host endocrine regulation of parasitoid development was investigated. Studies were performed in order to relate the critical period of A. suspensa larvae to host suitability. Larvae were ligated posterior to the ring gland and the time of pupation in portions of the larvae anterior and/or posterior to the ligature was noted. The critical period, after which enough ecdysone was secreted to initiate pupation, occurred 12-24 hr prior to the 7th day of larval development. Thus 5-day-old larvae were in the pre-critical period and 7-day-old larvae had passed the critical period. Two microliters of Altozar 53%, a juvenile hormone analogue (JHA) were injected into larvae at a dosage of 2.77µg of actual JHA per gram of mean larval weight. Five-day-old larvae were first treated and a second application was made 12-15 hr later. Larvae of similar age were topically treated with the same dosages of JHA. Peanut oil was used as solvent for injections and acetone for topical applications. Topically treated

and injected larvae pupated on the 8th and 9th days, respectively, while untreated controls pupated on the 7th day. JHA treated larvae gave rise to deformed pupae. JHA treated larvae were parasitized on the 7th day of larval development. Untreated 5- and 7-day-old larvae were exposed to parasitoids and served as controls. The duration of the parasitoid's egg stage in JHA treated larvae was not significantly different from that in 5-day-old untreated larvae. However, the duration of egg stage in untreated 7-day-old hosts was significantly longer. The percent parasitoid emergence was significantly different among treated and untreated controls. Percent parasitoid emergence was lowest in JHA injected and untreated 7-day-old hosts. Topically treated larvae yielded progeny at a rate which was lower than in 5-day-old hosts, but higher than for JHA injected or untreated 7-day-old larvae.

With respect to the influence of A. suspensa endocrines on B. longicaudatus development, it is possible that naturally secreted juvenile hormone by itself or in combination with ecdysone may affect the parasitoids directly or indirectly. The fact that the egg and pupal stages are affected is of interest since both these stages are known to undergo physiological and morphological changes and it is possible that these processes might have been disrupted by improper ratios of ecdysone and juvenile hormone which may occur in hosts that had passed the critical period. However, further research is needed to pin-point the precise physiological interrelationships between the host and parasitoid. Differences in effect of juvenile hormone analogues and naturally secreted juvenile hormone must also be established.

The number of eggs in the ovaries of female parasitoids deprived of hosts during their 21 day life time was determined. There was an increase in the number of eggs in parasitoid ovaries with an increase in parasitoid age up to 5 days of age after which there was a slight decline. The number of eggs in ovaries of females initially declined then increased again after a 24 hr oviposition period, indicating that B. longicaudatus females are 'synovigenic'. Females with 72 hr oviposition experience matured eggs at a faster rate than those with 24 hr oviposition. This indicated that frequent oviposition experiences stimulated females to mature more eggs.

The finding that B. longicaudatus females can discriminate and hence lay fewer eggs per host is of significance in a mass rearing program since mortality due to superparasitization is minimized. The determination of an optimum host age for parasitoid development further enhances production of large numbers of viable progeny. The implication that processes occurring during host maturation affect parasitoid development provides a basis for further investigation of the effect of host endocrines on parasitoid development. This could be investigated through in vitro study in which an artificial diet could be developed for rearing parasitoids. This medium could contain appropriate proportions of hormones to maximize parasitoid development. Further, the observation that female parasitoids with oviposition experience matured eggs throughout their life, could lead to the defining of an adult diet that would increase the viability and number of eggs produced, and extend the duration of the egg laying period of the parasitoids.

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BIOGRAPHICAL SKETCH

Pauline Olive Lawrence is the daughter of Mr. and Mrs. L. Lawrence of Buff Bay, Portland, Jamaica, West Indies. She was born on November 10, 1945. In 1957, she received a Jamaica Government Scholarship to attend the Titchfield Secondary School, Port Antonio, Jamaica where she passed the Senior Cambridge examination in 1962. She served as Head of the school's Student Body from 1963 to 1964, when she graduated after successful completion of the General Certificate of Education (Cambridge) examination, at the 'Advance Level'.

She attended the University of the West Indies, Mona, Jamaica from September 1964, to June 1968, where she majored in Zoology. She was awarded a scholarship by the Government of Jamaica from 1965 to 1968 when she received the Bachelor of Science degree (honors). From September 1968 to August 1969 she was employed by the Ministry of Agriculture (Plant Protection Division), Jamaica. In September 1969, she was granted a Jamaica Government scholarship to the University of Florida to pursue the Master of Science degree which she received in March 1972. She again enrolled at the University of Florida in September 1972 to pursue the Doctor of Philosophy degree. Pauline received a Graduate Research Assistantship as a means of support throughout her studies.

Pauline is a member of the Entomological Society of America, the Florida Entomological Society and the International Organization of

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

James L. Nation, Chairman
Professor of Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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